trically than in the case of *E. coli*. However, for some unknown reason, experiments with *B. subtilis* and *B. megaterium* have not borne out this expectation.

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Native and Renatured Transfer Ribonucleic Acid

Abstract. *Transfer ribonucleic acid, isolated under conditions in which the original macromolecular structure is never denatured, is indistinguishable from transfer ribonucleic acid prepared by conventional methods involving denaturing steps. This finding is consistent with the absence of direct genetic control of the formation of macromolecular structure of transfer ribonucleic acid.*

The reversibility of the denaturation of sRNA (*1*) in solution has been studied by a variety of methods. On cooling thermally denatured sRNA, complete recovery of hypochromicity and amino acid acceptor and transfer activities has been observed (*2, 3*). Solvent denaturation with 8*M* urea has also been found to be reversible (*3*).

In that a solution of sRNA in distilled water shows little or no hypochromicity (*4*) and a denaturing effect of phenol on polynucleotide helices has been noted (*5*), it is evident that conventional preparative methods for sRNA, involving phenol-water extractions, dialysis against distilled water, or exposure to elevated temperatures, lead at some stage to denaturation of the molecules. It has generally been presumed, however, that such denaturation is completely reversible. Thus, sRNA is not bound to proteins or any large molecules in cell-free extracts (*4*). Furthermore, heavy metals do not seem to be integral parts of the structure of sRNA (*6*). Nevertheless, it is apparent that studies on the reversible conformational transitions of sRNA have been made thus far with renatured, rather than truly "native" sRNA that has never undergone denaturation. This distinction would be of significance, were sRNA to be stabilized in vivo in a conformation that is not the lowest energy state in vitro.

For this work, a preparation of sRNA from yeast was made under conditions in which the original, macromolecular structure in vivo is presumably retained. Exposure to organic solvents, elevated temperature, or low ionic strength during the isolation process was avoided. The "native" sRNA so obtained has been compared on the basis of several physicochemical and biochemical criteria with "renatured" sRNA prepared by conventional procedures.

For the preparation of "native" sRNA, 170 g of bakers' yeast (harvested in the budding phase) were frozen, and ground with two parts of acid-washed glass beads (0.2 mm diameter) under liquid nitrogen. The material was then rapidly brought to 0°C and extracted for 30 minutes with 700 ml of a mixture of 0.01*M* sodium cacodylate, 0.004*M* $MgCl_2$, and 0.001*M* EDTA (Na), *p*H 6.2, in the presence of 4 mg of purified bentonite per milliliter (*7*). All subsequent purification steps were carried out at 2° to 4°C. After centrifugation at 13,000*g* for 30 minutes the extract was passed through a carboxymethylcellulose column (17 by 4.5 cm) equilibrated with the extracting solvent. The sRNA did not adsorb to the column. Solid NaCl was added to the eluate to a concentration of 0.05*M*, and the solution was immediately passed through a DEAE-cellulose column (19 by 2.2 cm). A linear NaCl gradient was used to elute the sRNA fraction, which appeared after 450 ml (Fig. 1). In order to concentrate the sRNA fraction, three volumes of a mixture of 0.01*M* sodium cacodylate and 0.002*M* $MgCl_2$, *p*H 6.2, were added, and the material was readsorbed onto a smaller DEAE-cellulose column (9 by 2.2 cm). The sRNA was then eluted with a mixture of 1.2*M* NaCl, 0.01*M* sodium cacodylate, and 0.002*M* $MgCl_2$, *p*H 6.2, in a volume of 50 ml. This solution was dialyzed against several changes of a mixture of 0.1*M* NaCl, 0.01*M* sodium cacodylate, 0.005*M* $MgCl_2$, and 0.0005*M* EDTA (Na), *p*H 6.85, for 18 hours, and frozen. For further studies dilutions were made with the last dialyzate. The yield was 100 to 110 mg of sRNA, an extinction coefficient at 22°C of A_{258} equal to 206 being used for 1 percent sRNA.

The preparation had a phosphorus content of 8.7 ± 0.2 percent and a protein content of < 2 percent. No loss of amino acid acceptor activity occurred after the preparation in solution was kept for 48 hours at room temperature, 22°C, an indication of the total absence of nucleases.

For comparison, sRNA isolated from commercial bakers' yeast by a procedure (*8*) involving denaturing steps such as phenol-water extractions and extensive dialysis against distilled water was used. This preparation, which has been extensively characterized and shown to be free from contaminants (*9*), was dissolved in a mixture of 0.1*M* NaCl, 0.01*M* sodium cacodylate, 0.005*M* $MgCl_2$, and 0.0005*M* EDTA (Na), *p*H 6.85 at room temperature and dialyzed against the same solvent at 2° to 4°C.

Absorption spectra of both preparations, recorded on a Cary 14 spectrophotometer at 22°C, were identical in the 220 to 300 $m\mu$ regions. Ratios for the absorption spectra were as follows: 220 $m\mu$/260 $m\mu$ = 0.67; 230 $m\mu$/260 $m\mu$ = 0.44; 280 $m\mu$/260 $m\mu$ = 0.47. Spectra in the interval from 310 to 370 $m\mu$ on more concentrated sRNA solutions (2 mg/ml) showed no absorption peaks, indicating the absence of significant amounts of thiopyrimidines in both preparations (*10*). Absorbance-temperature profiles at 260 $m\mu$ between 5° and 95°C, obtained in a thermostated Beckman DU spectrophotometer, also revealed no differences between the two sRNA preparations.

Sedimentation velocity experiments

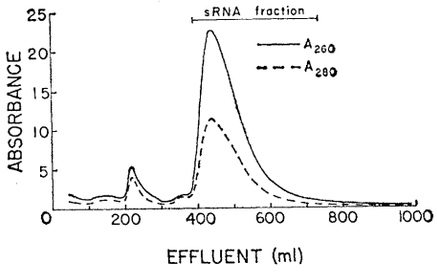

Fig. 1. Purification of sRNA by DEAE-cellulose chromatography. A linear gradient from a mixture of 0.1*M* NaCl, 0.01*M* sodium cacodylate, and 0.002*M* $MgCl_2$, *p*H 6.2 (500 ml), to a mixture of 1.2*M* NaCl, 0.01*M* sodium cacodylate, and 0.002*M* $MgCl_2$, *p*H 6.2 (500 ml) was used.

were made at 59,780 rev/min and 20°C in a Spinco E analytical ultracentrifuge. The sedimentation coefficients obtained with ultraviolet optics, at an sRNA concentration of 0.03 mg/ml, were, in Svedberg units, 4.21 ± .05 and 4.16 ± .05 for the "native" and "renatured" sRNA's, respectively. At concentrations of 2 mg of sRNA per milliliter no contaminating macromolecules in either preparation could be visually detected with schlieren optics.

Amino acid acceptor activity was determined for five amino acids (alanine, glycine, phenylalanine, tyrosine, and valine) (*11*), using a crude enzyme extract from yeast. After a prior incubation of the sRNA's in 1.5*M* tris buffer, *p*H 8.0, for 45 minutes at 37°C to remove amino acids bound to the sRNA (*12*), no increase in acceptor activity was detected for the "renatured" preparation, while small increases (10 to 25 percent) were obtained with the "native" preparation. A fraction of the "native" sRNA apparently retains terminal amino acids during the isolation procedure. However, no significant differences were found between the level of acceptor activities of the deacylated "native" and the "renatured" sRNA's. Moreover, the rate of aminoacylation with C^{14}-valine at 0°C was the same for the two sRNA's.

The kinetics and extent of reaction with formaldehyde (*13*) were also found to be the same within experimental error for the two sRNA preparations. Transfer RNA (1 mg/ml) was incubated with 0.3 percent C^{14}-formaldehyde. Portions (0.05 ml) were removed, first at 4-, later at 12-hour intervals, precipitated with 2 ml of cold 5 percent trichloroacetic acid, and filtered on to membrane filters; the precipitate was washed with 5 ml of 5 percent trichloroacetic acid, dried under an infrared lamp for 10 minutes, and counted in a scintillation counter. Varying either the volume of the wash fluid between 5 ml and 200 ml, or the drying time between 4 minutes and 25 minutes, produced no detectable differences in measured radioactivity.

The kinetics of degradation of sRNA to acid-soluble fragments by pancreatic ribonuclease differed slightly between the two sRNA preparations (Fig. 2). To explore the significance of this difference, "native" sRNA was heat-denatured, cooled, and again tested for nuclease sensitivity. No change in sensitivity was detected as a result of such heat denaturation and renaturation (Fig. 2). In additional studies on several phenol-extracted sRNA preparations, small but significant differences in nuclease sensitivity were observed even though these preparations exhibited indistinguishable physicochemical properties. Although the reason for the small variation in nuclease sensitivity of different sRNA preparations remains obscure, it does not seem to be correlated with conformational differences. Some of the individual sRNA molecules are more resistant towards enzymatic degradation than others (*11*), and the relative proportions of different amino-acid-specific sRNA's might vary slightly among different batches of yeast, depending on the yeast strains or the growth conditions.

In the absence of detectable differences between the "native" and "renatured" sRNA preparations investigated, it would appear that the native and renatured macromolecular structures of sRNA are identical. Apparent identity between native and renatured proteins has been described. Of special relevance to our work is the fact that proteins without disulfide bonds, such as *Bacillus subtilis* α-amylase (*14*) or yeast enolase (*15*), renature readily after complete denaturation even though there are no directing covalent bonds other than the peptide bonds. Such findings have been taken as evidence for the absence of direct genetic control of the formation of specific macromolecular structure of proteins (*16*). A similar conclusion has also been drawn for RNA molecules in general on the basis of theoretical considerations and experiments with synthetic polynucleotides (*17*). The present work

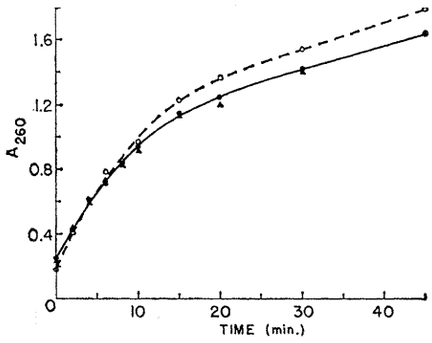

Fig. 2. Degradation of sRNA with pancreatic ribonuclease at 37°C. The sRNA concentration was 2 mg/ml; 1/50 volume of 0.5 μg ribonuclease and 2 mg of bovine serum albumin per milliliter were added. Portions were removed at different times, precipitated with four volumes of cold 5 percent perchloric acid, and centrifuged at 8000*g* for 5 minutes. The ultraviolet absorption of the supernatants was measured. A sample of the "native" sRNA was dialyzed against 0.001*M* EDTA, *p*H 6.5, for 24 hours, heated at 80°C for 10 minutes, cooled, and dialyzed back into the original solvent. Direct heat denaturation of the sRNA without these dialysis steps was not attempted, as Mg^{++} both stabilizes the secondary structure of sRNA and catalyzes the hydrolysis of phosphodiester bonds at high temperatures (*18*). Closed circle, "native" sRNA; closed triangle, the same preparation after the heat denaturation step; open circle, conventional "renatured" sRNA.

provides explicit confirmation of this for sRNA. Thus, the formation of macromolecular structure of sRNA appears to follow the same rules as for proteins, that is, the polymer chain searches out the conformation of lowest free energy, which is determined by the primary structure of the molecule.

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1. Abbreviations used: sRNA, transfer ribonucleic acid; DEAE, diethylaminoethyl-; EDTA, ethylenediaminetetraacetate; A_λ, absorbance at wavelength λ.
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Heterogeneous Catalysis: Effect of an Alternating Electric Field

Abstract. *Oxidation of carbon monoxide was studied in a static reactor at 123° ± 1°C, with NiO as catalyst. An alternating electric field of about 22,000 volts per centimeter, peak to peak, was applied normal to the catalytic surfaces. The rate of oxidation was thereby enhanced by a factor of 6 at 100 cycles per second, with maximum enhancement at between 100 and 200 cycles per second.*

I studied the oxidation of carbon monoxide in a static reactor containing 20 plates of grade-A nickel, 5.08 cm in diameter, oxidized by controlled oxidation to a thickness of about 1 μ NiO (*1*). The plates were separated by ceramic spacers—1-mm-thick rings. An a-c electric field of 22,000 volts/cm, peak to peak, was applied between the plates. At frequencies ranging from 50 to 300 cy/sec, the field consisted of a smooth sine wave (Fig. 1). The reactor was totally enclosed in a thermostat controlled at 123° ± 1°C. The reactant gases, air and CO, had been dried by passage through drying tubes containing 5A molecular sieve of 30 mesh; they were mixed before entering the reactor. The feed contained 0.028 percent CO_2 and 5.2 percent CO (both by volume); the balance was air. After purge of the reactor for 4 hours at a flow rate of 96 cm/min, the reactant mixture was trapped in the reactor at a pressure of 12.5 cm water above atmospheric.

Compositions of CO and air were determined by measurements of flow rates with two calibrated Matheson-610 flow meters. After a reaction period of from 19 to 68 hours, the resultant gaseous mixture was analyzed for CO_2 content by a gas chromatograph using a silica-gel column; the area under the gas-chromatographic curve was determined with a planimeter, with an estimated possible error of up to 0.01 percent (by volume) CO_2. Table 1 summarizes the data.

Net conversion of CO to CO_2 was the difference between CO_2 concentrations in the gaseous mixture before and after the reaction (Fig. 2). For the runs without electric field (runs 4/5, 4/6, and 4/12; Table 1), the relation was linear between the amount of CO converted to CO_2 and the resident time of the reactive mixture; this fact may suggest a zero-order reaction. However, since the change in CO concentration was relatively small, the order of the reaction cannot be asserted with certainty.

The net conversion to CO_2 during 19 hours in the reactor is plotted in Fig. 2 as a function of the a-c field frequency at a constant peak-to-peak field of 22,000 volts/cm. Apart from high-frequency distortions at 400 cy/sec, the field was a smooth sine wave. The enhancement of the rate of oxidation of CO does not reflect the heating effects of the a-c circuit because the capacitor plates, serving as catalyst, were the only part of the circuit in the reactor. *A fortiori*, the temperature of the reactor was automatically controlled at 123° ± 1°C during all experiments.

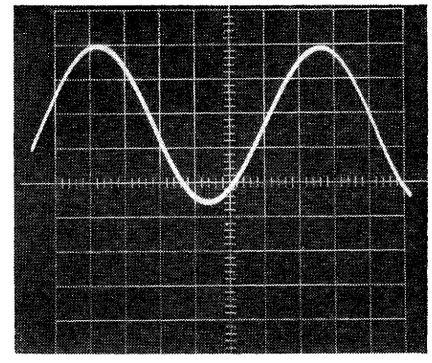

Fig. 1. Oscilloscope display of the sinusoidal electric field applied between the NiO catalytic surfaces. Scales: horizontal, 0.5 millisecond per division; vertical, 500 volts per division.

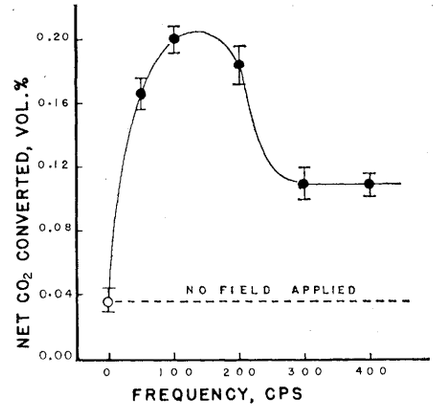

Fig. 2. Net increment (percentage by volume) of CO_2 after 19 hours in the reactor at 123° ± 1°C and at a pressure of 12.5 cm water above atmospheric. Open circle, with no field applied on the NiO catalyst; solid circles, with sinusoidal electric field applied. *Cps*, cycles per second.

The fact that the rate of reaction was maximum at between 100 and 200 cy/sec may imply that the resident time of the adsorbed species is of the order of 5 to 10 msec. The nature of the adsorbate and the reaction mechanism remain to be determined.

Table 1. Oxidations of CO to CO_2 during eight runs. PP, peak to peak.

Run	Duration (hr)	Field, PP (volts/cm)	Frequency (cy/sec)	CO_2 (vol %)	
				In residue	Net conversion
4/5	67	none		0.168	0.140
4/6	19	none		.060	.032
4/7	19	22,000	50	.195	.167
4/8	19	22,000	100	.230	.202
4/9	19	22,000	200	.215	.186
4/12	68	none		.175	.147
4/13	19	22,000	300	.138	.110
4/14	19	22,000	400	.138*	.110

* Wave form contained high-frequency distortions.

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2. I thank R. G. Schultz, M. A. Gilleo, and G. E. Barker of the Monsanto Co., St. Louis, Missouri, for their participation.

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